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GENETIC VARIATION IN *BOMBUS APPOSITUS* CRESSON (HYMENOPTERA: APIDAE)

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Abstract.—Genetic variation was measured in five populations of *Bombus appositus* Cresson. Scoring of 29 loci in 18 enzyme systems resulted in polymorphic loci in all populations and a low estimated mean heterozygosity of 0.028 ± 0.004 (SE). Neighbor-joining analysis did not resolve the geographic pattern of the populations.

Key Words.—Insecta, Hymenoptera, Apidae, population genetics, allozymes, *Bombus appositus*

Low levels of genetic variation have been reported in most Hymenoptera when compared to other insects (Graur 1985, Crespi 1991, Yik-Yuen et al. 1991). The exceptions are in Argidae and Tenthredinidae (Sheppard & Haydon 1986). This low level of variation is especially true for the bees (Apoidea) (Graur 1985; Pamilo et al. 1978, 1984; Owen 1985; Packer & Owen 1989; Scholl et al. 1990; Owen et al. 1992; Mullen & Rust 1994). Within the bumble bees (*Bombus* species) significantly low levels have been reported for 27 species (Pamilo et al. 1984, Owen et al. 1992) or about 10% of all *Bombus* species (Thorp et al. 1983). Heterozygosity estimates range from zero (0.0) in several species, *Bombus nevadensis* Cresson, *B. californicus* F. Smith, *B. rufocinctus* Cresson, *B. mixtus* Cresson, *B. perplexus* Cresson (Owen et al. 1992), to 0.044 in *Bombus balteatus* Dahlbom (Pamilo et al. 1984). Owen et al. (1992) found the mean expected heterozygosity for 16 *Bombus* species from North America to be low (0.008 ± 0.007 (SE)). Mullen & Rust (1994) found low (0.008 ± 0.004) but similar levels of heterozygosity in commercially reared and natural populations of *B. occidentalis* Greene.

There are several explanations for the low levels of genetic variation in *Bombus* (Pamilo & Crozier 1981; Graur 1985; Owen 1985, 1988; Owen et al. 1992). The degree of social evolution in Hymenoptera appears to be negatively correlated with genetic variability with the eusocial species having the lowest levels (Packer & Owen 1989).

This study examines the genetic variation within and between five populations of the bumble bee *Bombus appositus* Cresson. *Bombus appositus* was selected for study because it is member of the subgenus *Subterraneobombus* (Thorp et al. 1983). This subgenus contains only two species in North America *B. appositus* and *B. borealis* (Kriby) and has received no genetic study. Populations were sampled from the southern limits of the species range in the isolated mountain ranges of Great Basin Desert and the Rocky Mountain regions of western North America (Thorp et al. 1983).

MATERIALS AND METHODS

Female *B. appositus* were collected from three sites in northern Nevada and two sites in northern Utah (Table 1). Individual workers were collected from flowers while hiking (approximately 5 km distance) in the collection area over two or more days.

Table 1. *Bombus appositus* population sample sites (State, County, mountain range, latitude and longitude, and average elevation) and number of female individuals examined.

County	Population sites: Location	Latitude & Longitude	Elevation (meters)	Number
		Utah		
Cache	Wasatch Mountains			
	White Pine Lake	111°40′ × 41°55′	2500	21
Salt Lake	Wasatch Mountains			
	Mill Creek Canyon	111°40′ × 40°41′	2400	16
		Nevada		
Elko	Independence Mountains			
	Mill Creek	116°00′ × 41°31′	2500	28
Elko	Ruby Mountains			
	Thomas Creek Canyon	115°25′ × 40°38′	2500	22
Elko	East Humboldt Range			
	Angle Lake	115°05′ × 41°02′	2500	20

All field captured individuals were placed in individual plastic vials with cotton plugs and transported to the laboratory on ice and stored at −80° C. Individuals were prepared for electrophoresis by separating the head and thorax from the abdomen and homogenizing each in 0.05 ml of cold extraction buffer (Tris HCl 0.05 M, pH 7.0; May 1992). After 20 min of cold incubation and low-speed centrifugation, the supernatant was pipetted into 1.5 ml eppendorf tubes and stored at −80° C until used for electrophoresis. Supernatant was applied to 14% horizontal starch gels (50% Connaught and 50% Sigma) using filter-paper wicks (Whatman #3). Gels ran approximately five hours. All individuals were analyzed within two weeks of preparation. We used the methods and staining procedures described by May (1992). Twenty-eight enzyme systems were initially surveyed and 18 were used for population analysis.

Genotype frequencies were obtained by direct count from the phenotypes observed on the gels, and electromorph (allozyme) frequencies were calculated from genotype frequencies. All polymorphisms were photographed. The most common electromorph at each locus was designated as "C", with relatively faster migrating allozymes scored as "B" and relatively slower migrating allozymes scored as "D".

Expected genotypic frequencies, expected fit to Hardy-Weinberg equilibrium, Nei's unbiased expected heterozygosity and unbiased genetic identity (Nei 1978), and Wright's F-statistics (Wright 1978) were calculated using BIOSYS-1 (Swofford & Selander 1981). Neighbor-Joining unrooted tree construction (Saitou & Nei 1987) was produced with NJTREE (Jin & Ferguson 1990).

Results

Seven of the 28 enzyme systems (AAT, AC, ADH, LDH, ODH, SDH, XDH) yielded weak or no bands on the gels (Table 2). Two (GDH and G6PDH) were inconsistent or too smeared to score. CK was determined to yield the same banding pattern as AK and was dropped from scoring. The remaining 18 enzyme systems yielded 29 scorable loci (Table 2). Seven of the 18 enzyme systems

Table 2. Enzyme systems, loci, and electrophoretic conditions used to assay *Bombus appositus*, tagged enzymes (*) were used in the genetic analysis.

Enzyme (loci)	Symbol	E.C. Number[1]	Buffer[2]	Tissue[3]
Aconitase	AC	4.2.1.3	TC-1	A
Alcohol dehydrogenase	ADH	1.1.1.1	TC-1	A
*Adenylate kinase (1)	AK	2.7.4.3	TC-1	A
Aspartate aminotransferase	AAT	2.6.1.1	R	A
Creatine kinase	CK	2.7.3.2	4	T
*Diaphorase (NADH) (2)	DIA	1.8.1.4	4	T
*Diaphorase (NADPH) (1)	DIAP	1.8.1.4	R	A
*Esterase (2)	EST	3.1.1.–	4	T
*Galactosaminidase (1)	GAM	*******	C	A
*Glyceraldehyde-3-phosphate dehydrogenase (1)	GAPDH	1.2.1.12	C	T, A
Glucose dehydrogenase	GDH	1.1.1.118	TC-1	A
*a-Glycerophosphate (3) dehydrogenase	G3P	1.1.1.8	R	T, A
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49	TC-1	A
*Glucokinase (1)	GK	2.7.1.2	4	T
*General Protein (1)	GP	nonspecific	R	T
*Glucosephosphate (2) isomerase	GPI	5.3.1.9	R	T, A
*Hydroxybuteric (1) dehydrogenase	HBDH	1.1.1.30	TC-1	A
*Isocitrate dehydrogenase (2)	IDH	1.1.1.42	R	T
Lactate dehydrogenase	LDH	1.1.1.27	4	A
*Leucine aminopeptidase (2)	LAP	3.4.11.1	R	T, A
*Malic enzyme (2)	ME	1.1.1.40	4	T, A
*Malate dehydrogenase (1)	MDH	1.1.1.37	4	T, A
Octanol dehydrogenase	ODH	1.1.1.73	R	A
*Peptidase Leu-Ala (2)	PEP-LA	3.4.11	R	T, A
*Phosphoglucomutase (3)	PGM	5.4.2.2	4	T, A
Succinate dehydrogenase	SDH	1.3.99.1	TC-1	A
*Superoxide dismutase (1)	SOD	1.15.1.1	R	T, A
Xanthine dehydrogenase	XDH	1.1.1.204	R	A

[1] Nomenclature Committee International Union of Biochemistry 1984.
[2] Gel and tray buffer systems (May 1992).
[3] T = head and thorax, A = abdomen.

(DIAP, GAM, GAPDH, GP, IDH, LAP, MDH, and SOD) were fixed for the same allele in all individuals.

Table 3 contains the allele frequencies in the *B. appositus* populations sampled. The two Wasatch Mountains populations had both the fewest and most polymorphic loci, White Pine contained two (EST-2, PGM-2) and Mill Creek contained six (AK-1, GK-1, GPI-2, ME-1, PGM-1, PGM-2). In the Great Basin mountain populations, Ruby contained three (GK-1, GPI-2, PEP-1), Humboldt four (DIA-1, DIA-2, GPI-2, G3P-3), and Independence six (DIA-1, DIA-2, EST-2, GPI-2, PGM-2, PGM-3). All of the Humboldt and Ruby loci conformed to Hardy-Weinberg expectations. One locus each in the Independence (GPI-2, $\chi^2 = 19.06$, df = 1, $P = 0.000$) and White Pine (EST-2, $\chi^2 = 5.33$, df = 1, $P = 0.02$) samples and five loci in the Mill Creek samples (AK-1, $\chi^2 = 27.04$, df = 1, $P = 0.000$; GPI-2, $\chi^2 = 31.03$, df = 1, $P = 0.000$; ME-1, $\chi^2 = 31.03$, df = 1, $P = 0.000$; PGM-1, $\chi^2 = 31.03$, df = 1, $P = 0.000$; PGM-2, $\chi^2 = 31.03$, df = 1, $P = 0.000$) did not conform to Hardy-Weinberg expectations. All deviations were heterozygote deficiencies. Genetic variability was estimated within each sample with three mea-

Table 3. Allele frequencies at polymorphic loci and number of individual females analyzed in *Bombus appositus.*

Locus and alleles *n*		Utah		Nevada		
		White Pine 21	Mill Creek 16	Humboldt 20	Ruby 22	Independence 28
AK-1	B	0.000	0.062	0.000	0.000	0.000
	C	1.000	0.938	1.000	1.000	1.000
DIA-1	B	0.000	0.000	0.000	0.000	0.036
	C	1.000	1.000	0.975	1.000	0.964
	D	0.000	0.000	0.025	0.000	0.000
DIA-2	B	0.000	0.000	0.125	0.000	0.035
	C	1.000	1.000	0.875	1.000	0.965
EST-2	B	0.333	0.000	0.000	0.000	0.000
	C	0.667	1.000	1.000	1.000	0.938
	D	0.000	0.000	0.000	0.000	0.072
GK-1	B	0.000	0.031	0.000	0.000	0.000
	C	1.000	0.969	1.000	0.864	1.000
	D	0.000	0.000	0.000	0.136	0.000
GPI-2	B	0.000	0.062	0.050	0.000	0.000
	C	1.000	0.938	0.950	0.955	0.893
	D	0.000	0.000	0.000	0.045	0.107
G3P-3	B	0.000	0.000	0.025	0.000	0.000
	C	1.000	1.000	0.975	1.000	1.000
ME-1	C	1.000	0.938	1.000	1.000	1.000
	D	0.000	0.062	0.000	0.000	0.000
PEP-1	B	0.000	0.000	0.000	0.045	0.000
	C	1.000	1.000	1.000	0.955	1.000
PGM-1	C	1.000	0.062	1.000	1.000	1.000
	D	0.000	0.938	0.000	0.000	0.000
PGM-2	B	0.188	0.000	0.000	0.000	0.000
	C	0.812	0.938	1.000	1.000	0.965
	D	0.000	0.062	0.000	0.000	0.035
PGM-3	B	0.000	0.000	0.000	0.000	0.143
	C	1.000	1.000	1.000	1.000	0.857

sures: mean expected heterozygosity, percentage of polymorphic loci, and mean number of alleles per locus (Table 4). Mean expected heterozygosity ranged from 0.014 (Humboldt) to 0.03 (Independence). Percentage of polymorphic loci was low and ranged from 6.9% (White Pine) to 20.7% (Mill Creek and Independence). Mean number of alleles per locus was 1.1 or 1.2, in all populations.

A Neighbor-Joining phenogram (Saitou & Nei 1987) based on Nei's (1978) unbiased genetic identity (Table 5) was unresolved and did not represent the geographical relationships of the surveyed populations of *B. appositus* (Fig. 1). First, there was almost no separation of the populations (patristic values 0.0002) and branch length were identical (0.49). Second, the two Wasatch Mountain and three Great Basin mountain populations did not nest together; White Pine was associated with Humboldt (cycle 1), Mill Creek with Independence (cycle 3), and Ruby was placed between the two groups (cycle 2).

Hierarchical F-statistics for the 12 polymorphic loci indicate a high inbreeding coefficient within the populations (F_{is} = 0.382) and a moderate level (Hartl & Clark 1989) of differentiation among the populations (F_{st} = 0.105). The combined

Table 4. Genetic variability at 29 loci in all populations of *Bombus appositus*. Values in parentheses are standard errors.

Population	Mean number of alleles per locus	Percentage of polymorphic loci[1]	Mean expected heterozygosity[2]
		Utah	
White Pine	1.1 (0.0)	6.9	0.030 (0.021)
Mill Creek	1.2 (0.1)	20.7	0.024 (0.009)
		Nevada	
Humboldt	1.1 (0.1)	13.8	0.014 (0.008)
Ruby	1.1 (0.1)	10.3	0.015 (0.009)
Independence	1.2 (0.1)	20.7	0.025 (0.011)

[1] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99.

[2] Unbiased estimate (Nei 1978).

effects of nonrandom breeding and random genetic drift in the populations is also high (F_{it} = 0.447).

Discussion

The mean heterozygosity (H_{exp} 0.021 ± 0.011) in *B. appositus* is very low in relation to other Hymenoptera and insects (Graur 1985, Owen 1985, Crespi 1991). However, it is relatively high with respect to other species in the genus *Bombus*. Of the 27 species analyzed, only *B. balteatus* (0.044), *B. sylvicola* Kirby (0.042), *B. melanopygus* Nylander (0.037), *B. terrestris* (L.) (0.037), and *B. hypnorum* (L.) (0.025) have reported higher heterozygosity measures (Pamolo et al. 1984, Owen et al. 1992). The observed variation did not however provide any information on the genetic relatedness of the populations. High levels of inbreeding (F_{is} = 0.382) and the combined effects of inbreeding and random genetic drift were apparent in the populations (F_{it} = 0.447). These results reflect the primitive eusocial behavior of *Bombus* (Michener 1974, Brian 1983). They also suggest that the individual females sampled may have been sisters from the same colony, even though an attempt was made to obtain a broad sample of individuals from "many" colonies.

The Neighbor-Joining tree provided no information on the origin of the Great Basin mountain range populations with respect to the Rocky Mountains (Wasatch Mountains). The unresolved tree results from the lack of variation in the populations sampled.

Table 5. Matrix of genetic similarity of *Bombus appositus* Cresson populations as measured by Nei's (1978) unbiased genetic identity.

	Utah		Nevada		
Population	White Pine	Mill Creek	Humboldt	Ruby	Independence
White Pine	*****	0.998	0.997	0.997	0.998
Mill Creek		*****	0.999	0.999	0.999
Humboldt			*****	0.999	0.999
Ruby				*****	0.999
Independence					*****

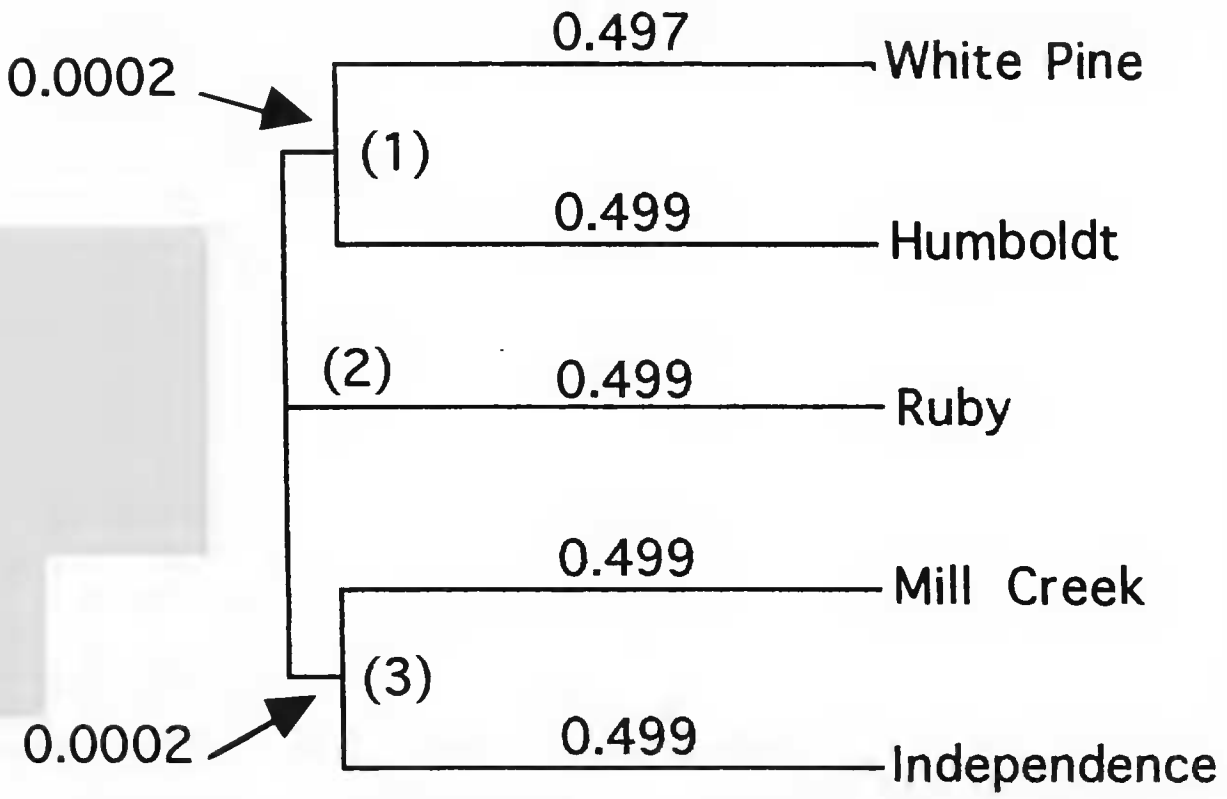

Figure 1. Neighbor-Joining tree of five populations of *Bombus appositus* Cresson based on Nei's (1978) unbiased genetic identities. Numbers along branches are branch lengths (patristic values) and numbers in parentheses are the analysis cycles. The tree is unresolved with basal branch lengths of essentially 0.0 (0.0002).

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